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Detection of BRCA1 and 2 Genes Mutations Among Women with Breast Cancer Attending Radio and Isotope Center in Khartoum, Sudan

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ABSTRACT

This study was conducted to estimate the frequency of Brca1 and Brca2 gene mutations in Sudanese female patients with breast cancer. One hundred patients (mean age 54.3 years) with breast cancer (BC) attending radio and isotopes center in Khartoum (RICK) together with 50 apparently healthy individuals (mean age 54 years), used as controls, were enrolled for this study. Among the patients, 63 have positive family history (1st and 2nd degree relatives) of BC. Brca1 & Brca2 genes were determined using specific primers and multiplied by PCR. The mutations of the 2 genes were detected on 2 exons (8 and 13) on Brca1 and exon 9 on Brca2 using single strand conformation polymorphism (SSCP) assay. The results showed no obvious mutation detected on both genes as indicated by silver polyacrylamide gel elecrophoresis. This study concluded that breast cancer on these patients might be predisposed by mutations on other genes or other exon on Brca1/2 and/or other risk factors.

المستخلص

اجريت هذه الدراسة لمعرفة التغير الوراثي فى جيني بركا 1 و بركا 2 لدى مئة من النساء المصابات بسرطان الثدي (بمتوسط عمر يعادل 54.3 سنة) و يترددن على مركز العلاج بالاشعة و النظائر المشعة بالخرطوم بالاضافة الى 50 امرأة سليمة استخدمت كمجموعة ضابطة. وجد ان هناك تاريخ للمرض لدى 63 امراة مصابة (صلة من الدرجة الاولى و الثانية). تم تحديد الجينين و مضاعفتهما باستخدام الية تضاعف الحامض النووي لمعرفة التغير فى هذا الجين و من ثم فحصت ثلاثة مناطق جينية (المنطقتان 8 و 11 من جين بركا 1 والمنطقة 9 من جين بركا 2) و ذلك باستخدام طريقة فحص الشريط المفرد لتاكيد التغير الجيني و الفصل الكهربائي باستخدام جلي الاكريل امايد المعالج بالفضة. اظهرت النتائج عدم وجود تغير فى الجينين. خلصت هذه الدراسة الى عدم وجود تغير جيني في المناطق التى فحصت من الجينين و يمكن ان يكون سرطان الثدي لدى هؤلاء النساء قد حدث لتغيير فى مواقع اخرى من الجينين او نتيجة لعوامل اخرى مسببة لسرطان الثدي.

KEYWORDS: breast cancer, susceptibility genes, genotyping, risk factors.

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INTRODUCTION

Breast cancer is the common malignancy in women worldwide, up to 12% of women are diagnosed as having breast cancer and about 3.5% of women die from this disease[1,2]. BC was known to be predisposed by several factors among which are mutations of tumor suppressor genes which increase the susceptibility on individuals to develop BC. These genes include high-risk breast cancer susceptibility genes (BRCA1, BRCA2, PTEN, TP53, LKB1/STK11 and CDH1) and low to moderate-risk breast cancer susceptibility genes (CHEK2, TGFβ1, CASP8 and ATM)[3]. The present work concerns with BRCA1 & 2 genes because these genes are associated with a high lifetime risk of breast cancer[4,5]. The risk of mutation of brac1&2 was currently estimated at 80% by the age of 70[6]. BRCA1 gene is expressed in the cells of breast and other tissue, where it helps repair damaged DNA, or destroys cells. If BRCA1 it self is damaged, damaged DNA is not repaired properly this will result in acquisition of deleterious mutations which increase individual's risk for developing cancers. BRCA1 is mapped to chromosome 17q21[7], it contains 24 exons and encodes a protein of 220 kDa, composed of 1863 amino acids[8]. The BRCA1 protein associates with RNA polymerase through the c-terminal domain, also interacts with histone deacetylase complexes. Thus, this protein plays a role in transcription, repair of breaks double – stranded DNA, ubiquitination, transcriptional regulation as well as other functions. BRCA1 mutation carriers have a 30% risk of developing ovarian cancer during their lifetime and
a 50–80% risk of developing breast cancer before the age of 70 years[9,10]. The BRCA2, is localized on the long arm of chromosome 13. BRCA2 is also a large gene, with 27 exons that encode a protein of 380 kDa, composed of 3418 amino acids[11]. Risk-associated truncation mutations are found throughout the entire BRCA1 coding sequence[12]. The majority of risk-associated mutations are frameshift or nonsense mutations that result in a premature stop codon and truncated protein product. Like other genetic mutations, BRCA1/2 mutations are rare in the general population. In U.S, between one of 400 and one of 800 people in the general population carry a BRCA1/2 mutation. However, prevalence varies by ethnic group. Among Ashkenazi Jewish men and women, about one in 40 individuals carries a BRCA1/2 mutation. In Sudan, BC was increasing on the last few years as indicated by the reports of the Radiation and Isotopes center in Khartoum, in which the numbers of 836 and 895 occurred among women in 2007 and 2008 respectively. These reports stated that BC in Sudan accounts for about 30% of all cancers in women. In addition to this reports, few researches in central Sudan were conducted. Among these, Awadelkarim et al. characterized germline BRCA1/2 mutations in BC patients under 40 years of age and concluded that mutation on these genes play an etiological role in BC in central Sudan[13, 14]. In another work, the prevalence of BRCA1&2 mutation was estimated to be 51% in those with a family history and 20% in those without family history of BC[13]. The aim of this study is to characterize the genetic mutations of BRCA1&2 in an attempt for prognosis of BC in Khartoum State.

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MATERIALS and METHODS

Study population

This study was conducted on 150 female subjects (100 patients with BC and 50 controls). All of them are Sudanese belonging to 13 tribes. Sixty three of the patients had positive family history of BC, 41 of them had BC in their 1st degree relatives and 22 had BC in their 2nd degree relatives. Ten of the 63 patients had ovarian cancer in addition to the BC. The mean age of the patients and control were 54.3 and 54 years, respectively.

DNA isolation and PCR amplification for the different exons

Blood samples (3 ml each) were collected from the patients and the controls in EDTA tubes. Genomic DNA was extracted from peripheral blood lymphocytes using a Promega DNA purification kit (Promega, Madison, USA), following the manufacturer's instructions. Universal primers (Table 1) were used to amplify two regions of the *BRCA1* gene (exons 8 and 13) and one region of *BRCA2* gene (exon 9). The polymerase chain reaction (PCR) was carried out using 50 ng of DNA, 10 × PCR buffer with 1.5 mM MgCl2, 2 µl of mixture of 4 mM dNTPs, 20 pmol of each primer and 1U of Tag DNA polymerase at final volume of 25 µl. The PCR conditions were 96°C for 5 minutes, then 35 cycles each consists of 30 sec at 94°C, one min at the annealing temperature of the primer used (56-59°C) and one min at 72°C, followed by one cycle at 72°C for 10 minutes.

Table 1: Primers' sequences employed in the specific-PCR
Primers Sequence (5'- 3')

BRCA	Exons	Sequence
1	8	Sense TGTTAGCTGACTGATGATGGT
		Antsense ATCCAGCAATTATTATTAAATAC
1	13	Sense AATGGAAAGCTTCTCAAAGTA
		Antsense ATGTTGGAGCTAGGTCCTTAC
2	9	Sense CATCACACTACTCAGGATGACA
		Antsense GCATGGTGGTGCATGCTTGTA

Mutation detection using the single strand conformation polymorphism assay (SSCP)

SSCP analysis was used to screen for mutations in the exons 8, 11 of *BRCA1* gene and exon 9 of *BRCA2* gene in all studied subjects. Following briefly the procedure described by Ibrahim et al 2010, every PCR product was mixed 1:1 with loading buffer (95% formamide, 0.05% bromophenol blue and 0.05% xylene cyanol), and denature at 98°C for 10 min and suddenly place in ice. Electrophoresis of the denatured PCR products were carried out in 8% polyacrylamide gel containing 5% glycerol and the run was performed at 30 mA constant current for 6 hours. After that, the gel was stained by silver for 10 minutes, washed by water and visualized using the gel documentation system[15].

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Statistical analysis

The data, either clinical or genetic findings, were statistically evaluated, interpreted and analyzed using SPSS version 11.5.

RESULTS

Distribution of study population among different tribes

Study population was distributed into 13 Sudanese tribes. The most frequent tribe was Jalaein with 43 individuals while the least frequent were Jaafra, Halfaween, Shuluk and Habania with one individual in each (table 2).

Table 2: Frequencies and percentages of patients according to the tribes

Tribes	Frequency	Percentage
Northern	80	80
Central	11	11
Western	4	4
Eastern	4	4
Southern	1	1
Total	100	100

Selection and amplification the exons on BRCA1&2 genes:

Exons 8 and 11 of BRCA1 and exon 9 of BRCA2 were detected using agrose gel electrophoresis that stained by ethidium bromide and 50bp DNA ladder as indicated in figures 1, 2 and 3.

1 2 3 DNA ladder

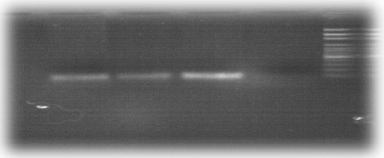

Figure 1: PCR product gel electrophoreses of BRCA1 (exon 8). Lanes 1, 2, 3 were samples and the 50 bp DNA ladder

1 2 3 4 DNA ladder

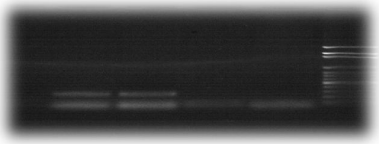

Figure 2: PCR product gel electrophoreses of BRCA 1 (exon13) . lanes 1, 2, 3 and 4 are samples. The right lane is 50 bp DNA ladder.

1 2 3 4 5 6 DNA ladder

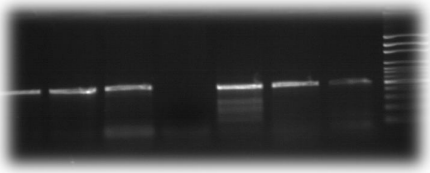

Figure 3: PCR product gel electrophoreses of BRCA2 (exon9). Lanes 1-6 are samples and 50 bp DNA ladder.

Detection of mutation using SSCP:

Mutations on the selected exons were screened by single strand conformation polymorphism assay but no mutation was seen on the silver stained polyacrylamide gel electrophoresis (figures 4 and 5**)**

DNA ladder 1 2 3 4 5 6 7 8 9 10

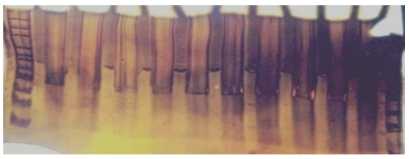

Figure 4: Exon 8 of BRCA1 seen as products of polyacrylamide gel electrophoresis for SSCP. Using 50 bp DNA, at the middle of the gel are the bands of the single strands located at the same level, indicating no variation among the single strands and at the front of the gel are the reannealed DNA strands. Lanes 1-10 are samples.

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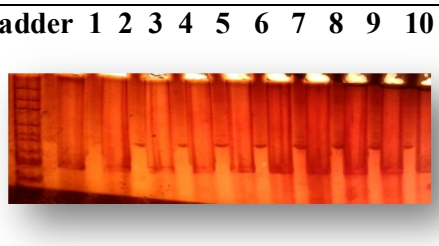

Figure 5: SSCP for BRCA2 (exon 9), seen as polyacrylamide gel electrophoresis. At the front are the reannealed DNA strands followed by the single strands which are located on the same level indicating no variation among the single strands. Ladder was 50 bp DNA.

DISCUSSION

Breast cancer is appearing to represent an enormous public health problem. The etiology of BC is thought to involve a complex interplay of genetic, hormonal and environmental factors that influence the\ physiological status of the host[16]. In this study, Promega kits were used to extract DNA from blood samples of all participants. Then, exons 8, 11 from BRCA1 and exon 9 from BRCA1&2 were selected and amplified using the specific primers (table 1). The selection of these exons was based on reports of previous works conducted on BC patients in Sudan, Egypt, some counties from Africa and other continents. The mutation in BRCA1 and BRCA2 genes were then assessed in patients with BC using SSCP assay. Identification of mutations in these genes was known to confer high risk of BC. The mutation in these genes was relevant for establishing preventive strategies for women with BC and prevention of contralateral breast or ovarian tumors. In addition, the detection of mutation in the patient's family allows identification of individuals at high risk, who can then seek genetic counseling[17]. In this study, BC was distributed among 14 Sudanese tribes with the northern tribes been populated with the highest number of BC patients (table 2). This can be explained on the fact that northern tribes are the most frequent tribes in the center of Sudan. However, no mutation was detected in the three exons selected on BRCA1&2 genes. This result will not rule out occurrence of a mutation in those genes on exon other than those assessed in this work. This result is in agreement with previous study conducted among Egyptian, Portuguese and Spanish BC patients[18]. This negative result can be explained on the basis that, there may be no inherited predisposition to the disease. The age of the patient at the diagnosis is an important risk factor for BC, as the risk increases steadily with age, so occurrence of BC in young age group gives strong implication for the presence of inherited genetic predisposition for BC[19,20]. In the present study, most of the BC patients acquired the disease at ages above 50 years, i.e. postmenopausal age. This result is in consistency with the postulation that patients who carry mutation on Brca1&2 develop the disease at earlier age, before 40 years and occurrence of BC in young age group indicates presence of inherited genetic predisposition for BC[5,19]. Women who are not likely to have inherited a BRCA mutation remain at risk of developing sporadic BC and women with putative inherited BRCA mutation confer an increased risk of developing BC[21,22]. However, this was inconsistent with our results as the frequency of the BC was higher in the old age group. This may be attributed to a possible impact of gene-environment interaction which delays the onset of the BC in the old age group. In this study, 61% of patients have positive family history with BC and ten of them BC accompanied with ovarian cancer. A positive family

history of BC usually reflects genetic susceptibility and it can be considered as one of the strongest risk factors for the disease[23, 24]. Our results indicate that a considerable proportion of the familial risk of BC is attributable to genes other than BRCA1&2 mutation[6, 15, 25]. In conclusion, our results indicated that BRCA1&2 mutation has a role in BC but a considerable proportion of the early BC and familial BC may be due to mutation on BRCA1&2 exons other than those screened in this study or genes other than BRCA1&2.

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